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Self-incompatibility and interspecific incompatibility: relationships in intra- and interspecific crosses of *Zinnia elegans* Jacq. and *Z. angustifolia* HBK (Compositae)*

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Summary. Intraspecific and reciprocal interspecific crosses involving *Zinnia angustifolia* clones and *Z. elegans* lines showed that in both species, sporophytic self-incompatibility (SI) systems were present. Intensity of SI varied among clones and lines, and high self seed set was associated with a concomitant decrease in callose fluorescence in papillae and pollen tubes. Incomplete stigmatic inhibition of pollen germination and tube growth was observed in reciprocal interspecific crosses and associated with callose synthesis, suggesting S-gene activity. Seed set and progeny obtained following *Z. angustifolia* × *Z. elegans* matings was comparable to intraspecific compatible matings of *Z. angustifolia* although the rate of pollen tube growth through the style was slower. In *Z. elegans* × *Z. angustifolia* matings, additional prezygotic barriers were present and acted between pollen tube penetration of the stigma and syngamy. SI × SI interspecific incompatibility was essentially unilateral, with no embryos or progeny obtained when *Z. elegans* was the pistillate parent. It was hypothesized that nonfunctioning of *Z. elegans* × *Z. angustifolia* crosses was due to S-gene expression at the stigmatic surface and to other isolating mechanisms in the stylar or ovarian transmitting tissue.

Key words: Incompatibility – Incongruity – Interspecific incompatibility – Ornamentals – Sporophytic incompatibility – Unilateral incompatibility – *Zinnia angustifolia* – *Zinnia elegans*

Introduction

The identification of breeding systems in cultivated plants and their near relatives is of fundamental importance for genotype selection and improvement. Self-incompatibility (SI), a genetically-controlled mechanism restricting inbreeding, is of interest to plant breeders for: permanent or temporary elimination of incompatibility to permit self-pollination; introduction of incompatibility into specific clones, cultivars or breeding lines from other lines or relatives; or utilization of incompatibility for hybrid seed production (Frankel and Galun 1977). The breeder interested in interspecific transfer of desirable traits may also encounter interspecific incompatibility (II) (de Nettancourt 1977) or incongruity (Hogenboom 1975), both of which are mechanisms preventing gene flow between species. Intra- and interspecific crossing relationships have been studied in two annual species, *Zinnia angustifolia* HBK (formerly *Z. linearis* Benth.) and *Z. elegans* Jacq., to determine whether these three mechanisms were present.

Zinnia (Compositae-tribe Heliantheae) is comprised of approximately 17 species, all native to the New World (Torres 1963). Selfing tests of *Zinnia* species indicated that SI is common in the genus, with 11 SI and 1 self-compatible (SC) species reported (Olorode 1970; Pollard 1939; Torres 1962, 1963, 1964). Self-incompatibility has been reported in *Z. angustifolia* (Olorode 1970) and *Z. elegans* (Pollard 1939), although conclusions were based on seed set and not on micro-

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scopic analysis of pollen germination or cytology of pollen tube growth.

In a previous study, 47.2% of *Z. angustifolia* florets and 4.6% *Z. elegans* florets examined contained embryos 14 days following reciprocal interspecific pollinations (Boyle and Stimart 1982). These results indicated that hybridization barriers existed in *Z. elegans* × *Z. angustifolia* matings but were not as restrictive in reciprocal pollinations. However, it was not determined whether II limits gene flow between these species by preventing pollen germination and/or restricting pollen tube growth.

The objectives of the present study were to elucidate the nature of barriers in intraspecific crosses of *Z. angustifolia* and *Z. elegans* and to determine if interspecific incompatibility acts as a pre-zygotic barrier to gene flow.

Materials and methods

All experiments were performed at the University of Maryland, College Park, MD (38°58′N latitude). Six lines of *Z. elegans* Jacq. (Canary Bird', 'Crimson Monarch', 'Enchantress', 'Orange King', 'Purity', and 'Scarlet Queen') and 4 clones of *Z. angustifolia* HBK (AO1, AW1, AW2 and AW9) were used as parents. Lines of *Z. elegans* were derived from partially-inbred commercial cultivars of the Giant Dahlia-Flowered series that were sib-crossed for 2 additional generations to increase uniformity of ray floret color. Clones of *Z. angustifolia* were selected at random from 2 commercial cultivars, with 1 cultivar nonsegregating for yellow florets (AO1) and the other cultivar segregating for yellow and white floret colors (AW1, AW2 and AW9).

Emasculation and crossing procedures were reported previously (Boyle and Stimart 1982). Following controlled pollinations, data were obtained on: 1) pollen germination (PG), pollen tube (PT) growth, and callose formation using the aniline blue fluorescence method; 2) kinetics of PT growth through the style; 3) presence or absence of embryos 3, 14 and 28 days following pollination; or 4) progeny testing.

For PG and PT analysis, a modification of Martin's (1959) procedure was used. Pistillate ray florets were pollinated and removed at 10 min intervals for the first hour, and at 24 h, and fixed in Carnoy's fluid (6 : 3 : 1 of 95% ethanol, chloroform and glacial acetic acid, respectively) for 24 h or longer. Florets were washed in water, and stained with 0.1% solution of aniline blue dissolved in 0.1 M K_3PO_4 for two or more hours. Callose resolution was optimum in pistils refrigerated at 3–4 °C for 24 h or more in 0.1% aniline blue solution. Styles were removed from ovaries, mounted in a drop of 0.1% aniline blue solution, and squashed under a cover slip. To estimate the number of ungerminated pollen grains and short PT following self-pollination of *Z. angustifolia* clones, styles were removed 24 h after pollination and mounted fresh in 0.1% aniline blue solution. A minimum of 10 styles were observed for each *Z. angustifolia* clone. Epifluorescence microscopy was performed using a Leitz SM-LUX microscope equipped with a Phillips 100 W high pressure Hg lamp. Measurements of pollen are those of the outside diameter inclusive of the spines and were taken with an ocular micrometer at 500 X on 15 random grains.

Pollen tube growth kinetics following intraspecific matings of *Z. angustifolia* and *Z. elegans,* and *Z. angustifolia* × *Z. elegans* matings, were studied by excising styles at the base above the ovaries at 15 min intervals for the first hour and at 24 h after pollination (Howlett et al. 1975). Embryological observations 7 days after pollination were used to indicate whether PT completed growth through the style in the allotted time interval, and were performed as described below in step 1 at 3 days.

Embryological observations 3 days after intra- and interspecific pollinations were obtained by: 1) excising ovules and removing the embryo sac under a dissecting microscope (40×); 2) squashing the embryo sac under a cover slip in a drop of 2% acetocarmine; and 3) viewing under a light microscope (100×). Embryological observations at 14 and 28 days after pollination were performed according to procedures outlined above in step 1 at 3 days.

Mature seeds from intra- and interspecific pollinations were harvested from inflorescences, dried at 20–23 °C and either sown immediately or stored at 3–4 °C for later use. Seeds were sown in flats filled with a soil-based medium and germinated under natural LD (14 h or more isolation) or natural daylengths plus supplemental incandescent irradiation (9 μmol s^{-1} m^{-2}) from 1600 to 2200 HR (Boyle and Stimart 1983). Clones of *Z. angustifolia* and selected inbreds of *Z. elegans* were asexually propagated by rooting terminal shoot cuttings under intermittent mist. Plants grown during fall, winter, and early spring received supplemental irradiation from 1600 to 2200 HR (12 μmol s^{-1} m^{-2}) from incandescent lamps. Greenhouse temperatures were maintained at a minimum of 15.5°/18 °C (night/day). Ventilation was provided automatically when air temperatures exceeded 24 °C.

In both species, single-gene markers controlling ray floret color (Boyle and Stimart 1985) were used for progeny testing to check for pollination-induced apomixis or contamination in controlled crosses. Confirmation of interspecific hybridization was based on morphological features of the progeny (Boyle and Stimart 1982) and chromosomal analysis (Boyle 1986).

Heterogeneity chi-square ($H X^2$) tests were used to test for differences among samples for percentage data (Little and Hills 1975).

Results

Zinnia angustifolia

Little or no callose in PT or stigmatic papillae was observed following cross-compatible pollinations (Fig. 1 a). Pollen grains, 20–21 μ in diameter, germinated 10–20 min after cross-compatible pollinations and produced PT approximately 30–60 μ in length before stigmatic penetration. The tube tip entered the style by penetrating at the papilla base. Stylar excision at 30 min after compatible pollinations indicated that pollen tubes in a majority of florets transversed the style within this time period and percent of florets with embryos did not increase by stylar excision at longer time intervals (Table 1).

Following self-pollination, intense callose fluorescence appeared in PT tips and papillae adjacent to pollen grains or PT (Fig. 1 b). Most pollen grains failed to germinate or produced short PT, often twisted or enlarged at the tip. Few (0–4) PT were observed attempting to penetrate AO1, AW2, and AW9 stigmas, despite heavy pollinations of approximately 200 grains/

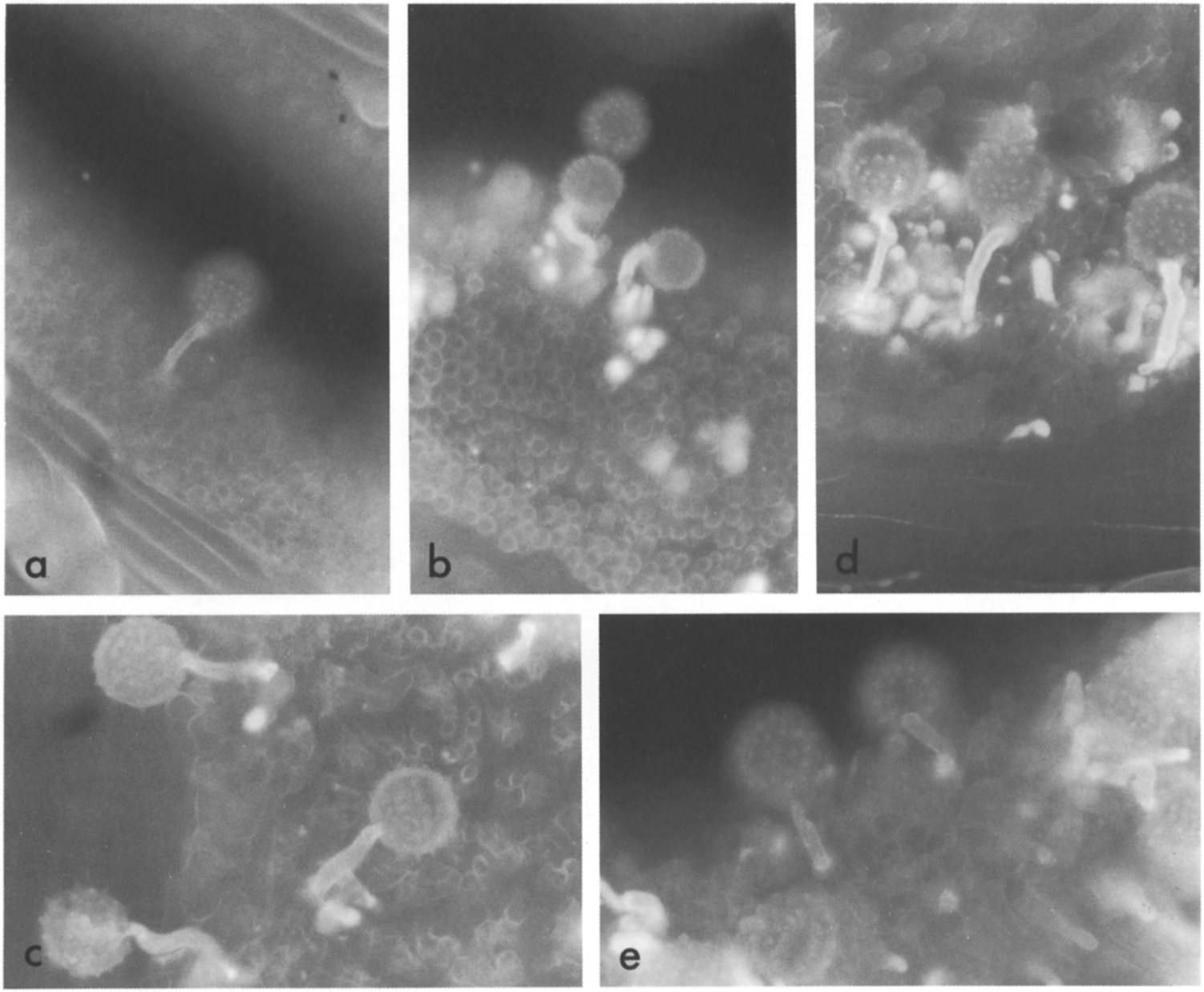

Fig. 1 a–e. Epifluorescence micrographs of pollen tubes on pistils of *Z. angustifolia* (*Z. a.*) clones and *Z. elegans* (*Z. e.*) lines stained with decolorized aniline blue. **a** *Z. a.* AW2 × *Z. a.* AW1, compatible mating; **b** *Z. a.* AW2 selfed, incompatible mating; **c** *Z. e.* 'Purity' × *Z. e.* 'Canary Bird', compatible mating; **d** *Z. e.* 'Orange King' selfed, incompatible mating, and **e** *Z. e.* 'Purity' selfed, compatible mating

Table 1. Effect of style excision after pollination on embryos observed 7 days after intraspecific compatible and interspecific matings of *Z. angustifolia* (*Z. a.*) and *Z. elegans* (*Z. e.*)

Cross	Time period after pollination (h)	Florets pollinated	Florets w/embryos (no.)	% florets w/embryos
Z. a. × *Z. a.*	0	24	0	0
	0.25	61	6	9.8
	0.5	49	42	85.7
	0.75	57	46	80.7
	1	62	55	88.7
	24	55	46	83.6
Z. e. × *Z. e.*	0	41	0	0
	0.25	36	1	2.8
	0.5	37	25	67.6
	0.75	49	36	73.5
	1	75	50	66.7
	24	39	30	76.9
Z. a. × *Z. e.*	0	26	0	0
	0.25	53	0	0
	0.5	56	21	37.5
	0.75	54	38	70.4
	1	58	37	63.8
	24	49	33	67.3

Table 2. Embryos observed 14 days after self-pollination of *Z. angustifolia* (*Z. a.*) clones. Experiment 1 performed in 1982 and Experiment 2 in 1983. Heterogeneity (H) X^2 values for the percentage of florets with embryos for Experiment 1 versus Experiment 2

Z. a. clone	Expt.	Florets pollinated	% florets w/embryos	H X^2
AO1	1	153	2.0	
	2	77	1.3	
	1+2	230	1.7	0.01 NS
AW1	1	58	29.3	
	2	113	22.1	
	1+2	171	24.6	1.10 NS
AW2	1	167	0.6	
	2	75	1.3	
	1+2	242	0.8	0.39 NS
AW9	1	150	4.0	
	2	93	8.6	
	1+2	243	5.8	2.17 NS

NS = Not significant: $P \geqq 0.05$

pistil. In contrast, up to 50 pollen grains per pollinated pistil were observed attempting to penetrate AW1. Callose deposition occurred in grains or PT adjacent to papillae but fluorescence was weaker generally than in other clones.

Percentage of florets with embryos 14 days following self-pollination ranged from 0.8% (AW2) to 24.6% (AW1) (Table 2). Results did not vary significantly within clones between experiments whereas variation among clones was significant (H $X^2 = 107.239$, $P < 0.005$).

Percentage of emerged seedlings from cross-pollinations among clones ranged from 68.1% to 80.6% and from self-pollinations the range was from 0% (AW9) to 30.9% (AW1) (Table 3). Variations among clones in percentage of emerged seedlings from self-pollinations was significant (H $X^2 = 61.105$, $P < 0.005$).

Progeny testing based on ray floret color of seedlings indicated that pollination-induced apomixis did not occur among the 4 *Z. angustifolia* clones.

Zinnia elegans

Cross-compatible pollinations were characterized by little or no callose in either PT or papillae (Fig. 1c), whereas in incompatible pollinations callose was prominent in PT and papillae. Pollen grains, 27–29 μ in diameter, developed PT approximately 12–53 μ before stigmatic penetration. Pollen germinated and PT penetrated the stigmatic surface 10–20 min after cross-compatible matings. Following compatible matings, pollen tubes had grown through the style in a majority of florets within 30 min after pollination (Table 1). 'Orange King' and 'Canary Bird' produced intense callose fluorescence in papillae and PT following self pollinations (Fig. 1d), and most pollen failed to germinate or produced short, curled or twisted PT. In contrast, callose fluorescence was not as intense or absent in PT or adjacent papillae of 'Crimson Mo-

Table 3. Seedlings obtained from self- and compatible cross-pollinations among 4 *Z. angustifolia* clones. Heterogeneity (H) X^2 values for percentage of emerged seedlings for self- versus cross-pollinations

Z. angustifolia clone	Self-pollinations		Cross-pollinations		H X^2
	Florets pollinated	% emerged seedlings	Florets pollinated	% emerged seedlings	
AO1	77	7.8	295	76.6	123.1***
AW1	97	30.9	274	80.6	51.5***
AW2	85	3.5	311	70.1	119.7***
AW9	113	0.0	292	68.1	120.4***

*** $P<0.001$

Table 4. Embryos observed 14 days following self- and compatible cross-pollinations of 6 *Z. elegans* lines. Heterogeneity (H) X^2 values for percentage of florets with embryos for self- versus cross-pollinations

Z. elegans line	Self-pollinations		Cross-pollinations		H X^2
	Florets pollinated	% florets w/embryos	Florets pollinated	% florets w/embryos	
'Canary Bird'	168	1.2	367	72.2	232.2***
'Crimson Monarch'	150	52.0	342	62.6	4.8*
'Enchantress'	127	50.4	357	58.5	2.5 NS
'Orange King'	154	18.2	320	70.0	112.3***
'Purity'	134	65.7	370	65.7	0.0 NS
'Scarlet Queen'	135	51.8	220	62.7	3.9*

NS = Not significant: $P \geqq 0.05$; * $P<0.05$; *** $P<0.001$

narch', 'Enchantress', 'Purity', and 'Scarlet Queen' following self-pollinations (Fig. 1 e).

Fewer embryos were observed following self-pollinations of 'Canary Bird', 'Crimson Monarch', 'Orange King' and 'Scarlet Queen' than following cross-pollinations whereas differences in self- and cross-pollinations were not significant for 'Enchantress' and 'Purity' (Table 4). Among the *Z. elegans* lines, significant variation existed in percentage of florets with embryos following self-pollination ($H X^2 = 197.725$, $P < 0.005$).

Progeny testing of *Z. elegans* lines using ray floret color markers revealed no pollination-induced apomixis.

Zinnia angustifolia × *Zinnia elegans*

Pollen germinated within 20 min after pollination and two types of PT growth were observed: 1) inhibited PT (curled or twisted) failing at stigmatic penetration and containing callose in the PT and adjacent papillae (typical of intraspecific incompatible matings) or 2) uninhibited stigmatic penetration of PT without callose in adjacent papillae or PT (typical of intraspecific compatible matings). Both inhibited and uninhibited PT were observed on each pistil (Fig. 2 a). Some pollen grains elicited callose in adjacent papillae although PT penetrated the stigma without callose deposition. Other pollen grains failed to germinate and produced callose in adjacent papillae. The callose rejection reaction in inhibited PT was usually weaker than in intraspecific incompatible matings.

Stylar excision after interspecific pollinations indicated that *Z. elegans* pollen tubes transversed *Z. angustifolia* styles in the majority of florets within 45 min (Table 1).

Embryos were observed 3, 14 and 28 days after pollination and 69.4–77.2% of florets contained embryos (Table 5, upper half). Variation among *Z. angustifolia* clones for mean percentage of florets with embryos was not significant and was not positively correlated with percentage of florets with embryos following selfing ($r = -0.53$). Percentage of germinated hybrid seedlings obtained from each *Z. angustifolia* clone was not affected significantly by the *Z. elegans* line used except for AW1 (Table 6); variation among *Z. angustifolia* clones was largely due to post-zygotic hybridization barriers (Boyle 1986). Comparisons of 'Canary Bird' (low self seed set) versus 'Purity' (high self seed set) revealed no differences in their fertiliza-

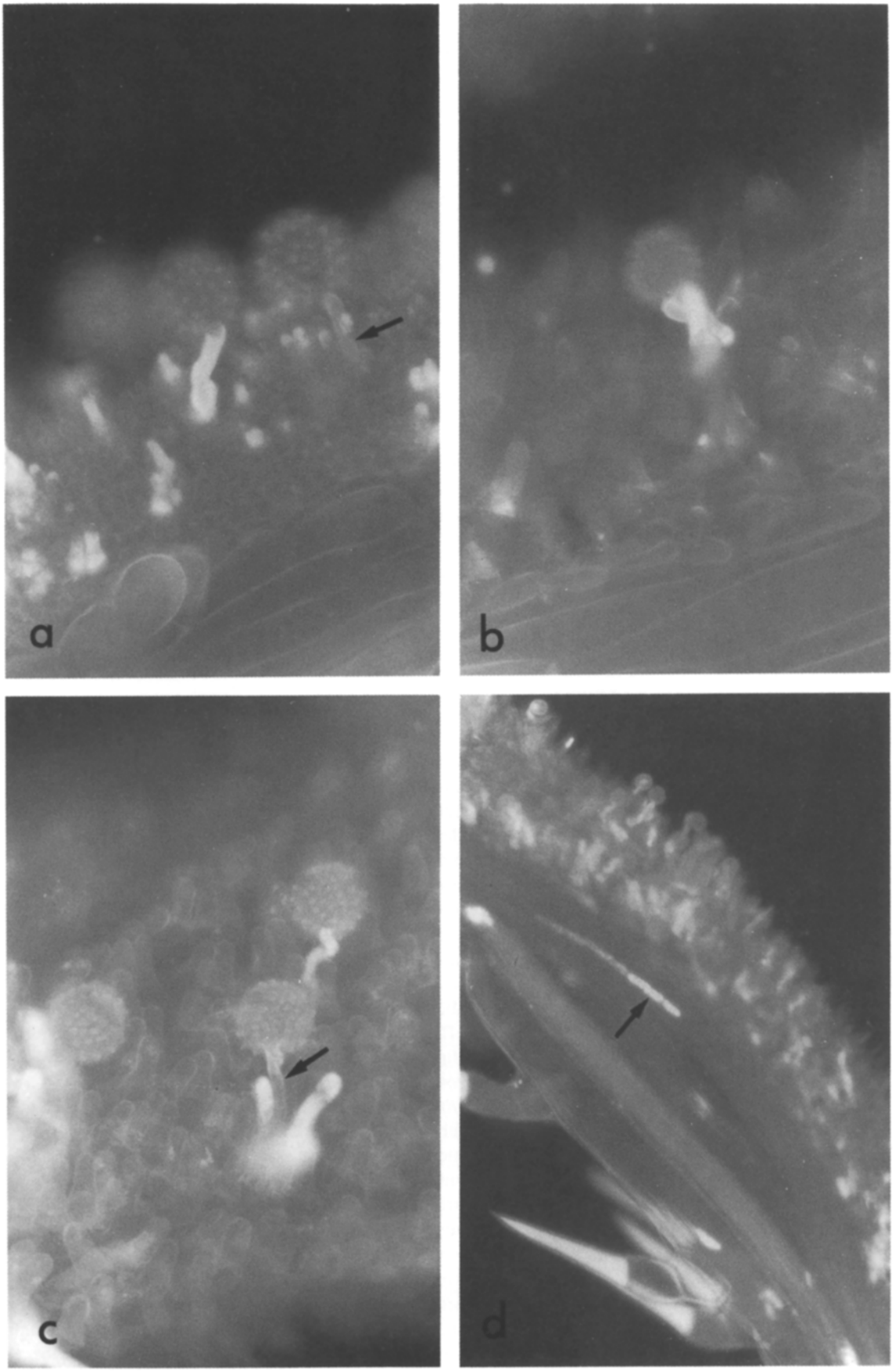
a
b
c
d

Table 5. Percentage of pollinated florets with embryos from reciprocal pollinations between 3 *Z. angustifolia* (*Z.a.*) clones and *Z. elegans* (*Z.e.*) 'Orange King'

Cross	Days after pollination						% florets w/embryos $\bar{X}$
	3		14		28		
	Florets pollinated	% florets w/embryos	Florets pollinated	% florets w/embryos	Florets pollinated	% florets w/embryos	
Z.a. AW1× *Z.e.* 'Orange King'	97	70.1	286	66.8	211	71.1	69.4
Z.a. AW2× *Z.e.* 'Orange King'	100	70.0	294	70.7	175	81.1	74.1
Z.a. AW9× *Z.e.* 'Orange King'	90	80.0	300	80.7	270	70.0	77.2
Significance[a]							1.78 NS
Z.e. 'Orange King' ×*Z.a.* AW1	93	0	286	0.4	–	–	0.3
Z.e. 'Orange King' ×*Z.a.* AW2	100	0	313	0	–	–	0
Z.e. 'Orange King' ×*Z.a.* AW9	100	0	131	0	–	–	0

[a] Differences not significant (NS) at 5% level by F test on arcsin transformed data

tion capacity for producing interspecific hybrids (Table 7). Morphological and chromosomal analysis of progeny from *Z. angustifolia*×*Z. elegans* matings confirmed interspecific hybridization (Boyle 1986).

Zinnia elegans×*Zinnia angustifolia*

As observed for the reciprocal cross, PT showed either inhibited or uninhibited growth at the stigmatic surface (Fig. 2, b and c). Lines of *Z. elegans* displaying high self seed set had generally more germinated pollen grains and pollen with uninhibited PT than *Z. elegans* lines with low self seed set. Stigmatic responses of PT did not vary among the *Z. angustifolia* clones used as pollen parents. Aberrant stylar growth of PT was observed when some plants of *Z. elegans* 'Scarlet Queen' were used as female parents (Fig. 2d). Examination of florets 3 and 14 days after pollination indicated less than 1% with embryos (Table 5, lower half), and was independent of the *Z. angustifolia* clones used as pollen parents (HX^2 [at 3 days]=0.00, $P=1.00$; HX^2 [at 14 days]=1.5, $P=0.50$–0.30).

Discussion

Observations using epifluorescence microscopy, supported by embryological and seedling emergence data, indicated SI systems in *Z. angustifolia* and *Z. elegans* and confirmed earlier reports of SI in these species based on seed set (Olorode 1970; Pollard 1939). In addition, stigmatic callose rejection reactions following self-pollination strongly suggested sporophytic control of SI for both species (Heslop-Harrison et al. 1973).

Sporophytic control of SI has been reported for many Compositae genera, including: *Ageratum houstonianum* (Stephens et al. 1982); *Carthamus flavescens* (Imrie and Knowles 1971); *Chrysanthemum carinatum* (Jain and Gupta 1960), *C. cinerariaefolium* (Brewer 1974) and *C. morifloium* (Zagorski et al. 1983); *Cichorium intybus* (Eenink 1981); *Cosmos bipinnatus* (Crowe 1954); *Crepis foetida* (Hughes and Babcock 1950); *Helianthus annuus* (Habura 1957); and *Parthenium argentatum* (Gerstel 1950). Genetic analysis of SI systems in *Z. angustifolia* and *Z. elegans* are currently in progress and will resolve whether SI is sporophytically controlled in these species.

Percentage of florets with embryos following self-pollinations varied significantly among clones of *Z. angustifolia* and

Fig. 2a–d. Epifluorescence micrographs of pollen tubes on pistils of *Z. angustifolia* (*Z.a.*) and *Z. elegans* (*Z.e.*) stained with decolorized aniline blue. **a** *Z.a.* AW2×*Z.e.* 'Scarlet Queen', inhibited (*left*) and uninhibited (*right, arrow*) pollen tubes; **b** *Z.e.* 'Scarlet Queen'×*Z.a.* AW1, inhibited pollen tube and papillae with callose deposition; **c** *Z.e.* 'Orange King'×*Z.a.* AW9, uninhibited pollen tube (*arrow*) and callose in adjacent papillae; **d** *Z.e.* 'Scarlet Queen'×*Z.a.* AW1, aberrant pollen tube growth in style (*arrow*)

Table 6. Interspecific hybrids obtained from *Zinnia angustifolia* × *Z. elegans* crosses. Heterogeneity (H) X^2 values for seedling emergence percentage

Female parent	Pollen parent	Florets pollinated	Seedlings (no.)	% emerged seedlings
AO1[a]	'Orange King'	130	48	36.9
	'Canary Bird'	132	42	31.8
	'Crimson Monarch'	98	38	38.7
	'Enchantress'	–	–	–
	'Purity'	88	32	36.4
	'Scarlet Queen'	–	–	–
		448	160	35.7
AW1[b]	'Orange King'	72	24	33.3
	'Canary Bird'	132	51	38.6
	'Crimson Monarch'	108	26	24.1
	'Enchantress'	96	12	12.5
	'Purity'	72	25	34.7
	'Scarlet Queen'	559	132	23.6
		1,039	270	26.0
AW2[c]	'Orange King'	133	75	56.5
	'Canary Bird'	228	115	50.4
	'Crimson Monarch'	145	78	53.8
	'Enchantress'	121	57	47.1
	'Purity'	97	46	47.4
	'Scarlet Queen'	569	307	53.9
		1,293	678	52.4
AW9[d]	'Orange King'	45	3	6.7
	'Canary Bird'	31	3	9.7
	'Crimson Monarch'	28	2	7.1
	'Enchantress'	25	2	8.0
	'Purity'	27	4	14.8
	'Scarlet Queen'	–	–	–
		156	14	9.0

[a] H $X^2 = 1.36$. $P = 0.75$–0.50
[b] H $X^2 = 26.76$. $P < 0.005$
[c] H $X^2 = 4.20$. $P = 0.75$–0.50
[d] H $X^2 = 1.59$. $P = 0.90$–0.75

Table 7. Interspecific hybrids obtained from *Zinnia angustifolia* × *Z. elegans* crosses. Heterogeneity (H) X^2 values for seedling emergence percentage for *Z. elegans* 'Canary Bird' versus 'Purity'

Female parent	Self seed status	Pollen parent	Self seed status	Florets pollinated	% emerged seedlings
AO1[a]	Low	'Canary Bird'	Low	132	31.8
		'Purity'	High	88	36.4
AW1[b]	Med	'Canary Bird'	Low	132	38.6
		'Purity'	High	72	34.7
AW2[c]	Low	'Canary Bird'	Low	228	56.4
		'Purity'	High	97	47.4
AW9[d]	Low	'Canary Bird'	Low	31	9.7
		'Purity'	High	27	14.8

[a] H $X^2 = 0.49$, $P = 0.50$–0.25
[b] H $X^2 = 0.30$, $P = 0.75$–0.50
[c] H $X^2 = 0.26$, $P = 0.75$–0.50
[d] H $X^2 = 0.34$, $P = 0.75$–0.50

lines of *Z. elegans*. Variability in the SI response (based on seed set) has been reported for other Compositae genera: *Ageratum* (Stephens et al. 1982), *Chrysanthemum* (Ronald and Ascher 1975), and *Cichorium* (Eenink 1981). Whether the limited seed set of *Z. angustifolia* clone AW1 and *Z. elegans* 'Crimson Monarch', 'Orange King' and 'Scarlet Queen' was due to pseudo-self-compatibility (i.e., self seed set by a normally SI plant) is not known; progeny of these individuals were not tested for presence of a fully functional SI system (Ascher 1976). *Zinnia elegans* 'Enchantress' and 'Purity' set seed following self-pollination at levels comparable to outcross pollinations and were apparently SC. Self compatibility in *Z. elegans* has resulted probably from selection for maximum seed set during domestication, with SC genotypes segregating during inbreeding. Incompatibility alleles conferring strong SI responses and low self seed set may be useful for exploitation in hybrid seed production of *Z. elegans*.

Stigmatic callose rejection reactions following self-pollination were correlated negatively with percentage of florets with embryos for both species. It seems therefore that a decrease in strength of the SI response is associated with a concomitant reduction in callose in papillae and PT. A similar pattern was observed in *Brassica oleracea* following self-pollinations: callose developed in PT of a highly SI line and little or no callose occurred in PT of a weakly SI line although callose was present in stigmas of both lines (Kerhoas et al. 1983). These observations support the hypothesis that stigmatic callose production in sporophytic SI systems is a response to S-gene expression and self-recognition (Dumas and Knox 1982).

Fluorescence microscopy utilizing aniline blue as an indicator of callose is a convenient and rapid method for assaying the relative strength of SI alleles in *Z. elegans* lines and may have applications in other cultivated Compositae species. However, this method is limited to qualitative analysis via the relative intensity of callose fluorescence and is not a protocol for quantitative analysis of callose (Dumas and Knox 1983).

Callose was not detectable in PT in stylar transmitting tissue following intraspecific compatible matings in *Z. angustifolia* and *Z. elegans*. Similar observations have been reported for other Compositae genera: aniline blue fluorescence of PT was faint in *Cosmos bipinnatus* (Knox 1973) and *Helianthus annuus* (Vithanage and Knox 1977), and not detected in *Cichorium intybus* (Eenink 1981) and *Chrysanthemum morifolium* (de Jong and Kho 1982). In many species, both SC and SI, a succession of callose plugs develop in elongating PT which allow quantitation of tube growth in terms of distance traveled and the number of PT in different regions of the pistil (Dumas and Knox 1973). Quantitation of compatible tube growth in *Z. angustifolia* and *Z. elegans* has not proved possible using aniline blue fluorescence. The question remains: Is the virtual absence of callose in PT a regular feature of Compositae, and what is its physiological and evolutionary significance?

Interspecific incompatibility (II) has been reported as a breeding barrier between many Angiosperm species (de Nettancourt 1977). A variant of (II) commonly observed in interspecific crosses is unilateral incompatibility, occurring when pollen of 1 species is inhibited from functioning on pistils of another while no inhibition occurs for the reciprocal cross (Lewis and Crowe 1958). Lewis and Crowe (1958) observed that unilateral incompatibility usually occurred when SI species were pollinated by SC species. Intergeneric crosses among Cruciferae species (sporophytic SI) supported the SI×SC incompatibility hypothesis, with SI×SI crosses reported as compatible (Lewis and Crowe 1958). In contrast, Sampson (1962) found incompatibility in the majority of crosses among 10 SI Cruciferae species, suggesting that SI×SI inhibition may operate in families with sporophytic systems.

Results of this study indicated that SI×SI inhibition occurred in crosses between *Z. angustifolia* and *Z. elegans*, and embryological studies demonstrate that II was essentially unilateral with pre-zygotic inhibition occurring with *Z. elegans* as the female parent. Seed set following interspecific mating with *Z. angustifolia* as the female parent was comparable to intraspecific compatible crosses within *Z. angustifolia*, despite an apparent slower rate of PT growth through the style (Table 1). This suggests that *Z. elegans* PT in stylar and ovarian transmitting tissue of *Z. angustifolia* are not inhibited from ultimately reaching the embryo sac. These results confirm an earlier study indicating that hybridization barriers exist in *Z. elegans*×*Z. angustifolia* matings but were less strigent in reciprocal pollinations (Boyle and Stimart 1982).

Unilateral failure of crosses between *Z. angustifolia* and *Z. elegans* are in part due to pre-fertilization processes that occur when *Z. angustifolia* PT penetrate the stigma and interact within pistils of *Z. elegans*.

Pre-fertilization processes which regulate PT growth may be physiological or morphological in nature. Pollen tube growth in vivo appears to be partly heterotrophic and maternal tissue may provide respiratory substrates, minerals, specific structural compounds (or their precursors), enzymes and/or hormones (Rosen 1971). Aberrant PT growth may result from a physiological inability of PT to metabolize substrates or promoters in maternal tissues that are required for normal growth, as has been suggested for interspecific crosses in *Lilium* (Ascher and Peloquin 1968). Alternatively, PT of one species may lack genetic information to penetrate barriers or overcome inhibitors in the pistil of the other species (Hogenboom 1975). Unilateral failure in interspecific crosses may also result from longer stylar and ovarian transmitting tissue in one species which would function as an isolating mechanism to gene exchange (Stebbins 1950). This phenomenon has been reported for crosses between *Zea mays* and *Tripsacum* species (Allard 1960), and interspecific crosses in *Nicotiana* (Allard 1960), *Lilium* (Ascher and Peloquin 1968), and *Rhododendron* (Kho and Baer 1973). Discrete differences in transmitting tissue length were observed in the two *Zinnia* species used in this study. *Zinnia elegans* is approximately 18.4 mm from the junction of the two stylar arms to the base of the embryo sac whereas *Z. angustifolia* is ca. 6.1 mm (means of 5 pistils of each species). Additional microscopal research is needed to characterize stylar and ovarian barriers in *Z. elegans*×*Z. angustifolia* matings.

Inhibited PT growth following reciprocal crosses between *Z. angustifolia* and *Z. elegans* was associated with callose rejection reactions in PT and adjacent stigmatic papillae. Interspecific and intergeneric matings in Cruciferae (Sampson 1962; Kerhoas et al. 1983) and other Compositae genera (Vithanage and Knox 1977) also displayed stigmatic rejection responses associated with callose deposition in PT and papillae. The response phenotype was similar to the SI reaction, and this observation has been cited as evidence for S-gene involvement in II (Dumas and Knox 1983). In contrast to SI systems, II is not usually S-allele specific but occurs among all pistils of 1 species and pollen of another (Hogenboom 1975; Lewis and Crowe 1958). Our data support the contention that

II lacks S-allele specificity: 1) inhibited PG and PT were observed on all stigmas following reciprocal crosses between *Z. angustifolia* and *Z. elegans*; and 2) no relationship existed between the number of hybrid embryos or progeny obtained and the SI reaction of *Z. angustifolia* clones or *Z. elegans* lines. Although these observations do not indicate conclusively that SI and II are unrelated, they do not suggest that all S-alleles code for a common sequence that plays a role in interspecific recognition if in fact S-gene activity is involved in II.

Two issues are central to the discussion of interspecific incompatibility: 1) the stage of determination at which pollen receives the II phenotype, i.e., gametophyte or sporophyte; and 2) the presence or absence of S-gene activity in determining the II reaction. Three main groups of hypotheses have been put forward to address the latter issue; 1) II and SI are controlled by different elements of the S-locus; 2) II is controlled by the S-locus with additional major genes or polygenes acting as rejectors in the pistil of certain pollen phenotypes; and 3) II is not a function of S-locus activity but controlled by rejection genes or the absence of suitable genetic information. Hogenboom (1975) used "incongruity" to describe interspecific incompatibility not due to S-gene expression but caused by the absence of suitable genetic information. Incongruity, a product of evolutionary divergence, is expressed as non-functioning of pollen-pistil relationship due to physiological and/or morphological differences between two species (Hogenboom 1975). An alternative hypothesis, based on phenotypic observations in this study, is that interspecific hybridization of *Z. elegans* × *Z. angustifolia* may be prevented due to S-gene expression at the stigmatic surface and to other isolating mechanisms acting between PT penetration of the stigma and syngamy. Thus, non-functioning of the pollen-pistil relationship may not be due solely to incongruity and to the exclusion of S-gene expression. Genetic analysis of the SI and interspecific incompatibility systems may further elucidate factors involved in intra- and interspecific relations between *Z. angustifolia* and *Z. elegans* and determine whether S-gene activity is involved in expression of II.

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